THE EMBRYO SAC AND EMBRYO OF *GNETUM GNEMON*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 112

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(WITH PLATE VII)

Gnetum Gnemon has been made conspicuous among the other species of Gnetum chiefly by the investigation of LOTSY published in 1899.[1] The structures described were of such interest that it seemed desirable to supplement the somewhat incomplete account by a further examination. Accordingly material was obtained from the Philippine Islands and from British Guiana; the former from Dr. H. N. WHITFORD, and the latter from Mr. A. W. BARTLETT, director of the Botanic Garden at Georgetown. This material was first assembled by Dr. W. J. G. LAND, of this laboratory, in connection with his investigation of Ephedra; and he has kindly turned it over to me for separate study. The preparations and drawings were made by Dr. SHIGÉO YAMANOUCHI, of this laboratory, and to his technical skill the results are largely due.

The material included stages from two successive seasons, but unfortunately many intervening stages were not represented, so that no continuous account can be given. However, certain facts have been discovered that supplement and correct the previous accounts.

EMBRYO SAC

LOTSY described the embryo sac of *G. Gnemon* as showing an interesting deviation from those found by KARSTEN[2] in other species of Gnetum. Instead of containing only free nuclei at the fertilization stage, the embryo sac of *G. Gnemon* was described as containing

[1] LOTSY, J. P., Contributions to the life history of the genus Gnetum. Ann. Jard. Bot. Buitenzorg II. 1:46–114. *pls. 2–11.* 1899.

[2] KARSTEN, H., Bot. Zeit. **50**:205–215, 221–231, 237–246. *pls. 5, 6.* 1892; Ann. Jard. Bot. Buitenzorg **11**:195–218. *pls. 17–19.* 1893; Cohn's Beitr. Biol. Pflanzen **6**: 337–382. *pls. 8–11.* 1893.

a compact antipodal tissue, sharply distinct from the micropylar chamber with its free nuclei. As a consequence, the embryo sac of *G. Gnemon* has been used ever since as illustrating a female gametophyte intermediate in structure between the tissue-filled sacs of Ephedra and Tumboa on the one hand, and the sacs of other species of Gnetum, which contain only free nuclei. Later the same investigator in reporting parthenogenesis in *G. Ula*[3] described the embryo sac of that species as being of the *G. Gnemon* type.

Our material of *G. Gnemon* does not confirm this account. At an early stage of the embryo sac, eight nuclei are observed grouped near the center (*fig. 1*), the sac being invested by the loose tissue of the nucellus. At a somewhat later stage the nucellar cells at the chalazal end of the sac are strikingly differentiated (*fig. 2*), becoming more and more compactly arranged, gradually obliterating the intercellular spaces, and taking on the appearance of glandular cells. The relation of this tissue in its early stage to the embryo sac is shown in *fig. 2a*. As vacuolation proceeds in the sac and the free nuclei become parietally placed, this "pavement tissue" becomes more compact and extends deeper into the chalaza (*figs. 3, 3a*). Still later it spreads laterally below, until it becomes fan-shaped in section (*figs. 4, 4a*), but it is always very distinct in contour and sharply marked off from the surrounding nucellar tissue. At the fertilization stage (*figs. 4, 5*) the sac contains only free nuclei, which become somewhat grouped at the antipodal end (*fig. 5*), but there is no walled tissue. Spreading below the sac, however, the mass of nucellar pavement tissue shows a definite contour, which might be merged in imagination with that of the sac and thus mistaken for a compact tissue within the antipodal end of the sac. LOTSY's figures show the real contour of the sac, and his antipodal tissue is clearly this glandular pavement tissue developed in the chalaza. So far as the sac of *G. Gnemon* is concerned, therefore, its fertilization stage is that described for other species of Gnetum. It will be noted that after the fertilization stage is reached (*fig. 5*) the pavement tissue begins to lose its glandular character; and later it is destroyed entirely by the growing endosperm.

[3] LOTSY, J. P., Parthenogenesis bei *Gnetum Ula* Brongn. Flora 92:397–404. *pls. 9, 10.* 1903.

ENDOSPERM

A year later, the endosperm has destroyed all of the nucellar tissue except a very small amount at the tip (*figs. 6, 6a, 7a*), and it is clearly differentiated into a central region of smaller, more compact cells, and a more extensive peripheral region of larger and looser cells. In destroying the nucellar beak, a curious result is observed. The central region of the endosperm advances into the beak and then spreads laterally (*fig. 6*). In the meantime the peripheral region advances more slowly toward the beak, and as a consequence a ring of nucellar tissue is pinched between two growing masses of endosperm. The growth of the endosperm into the chalazal region also results in pressure toward the beak, so that the pinched nucellar tissue is under considerable pressure and becomes completely disorganized. Under this pressure some of the adjacent endosperm cells also become disorganized.

In ovules of the preceding year, at the fertilization stage of the embryo sac, a curious disorganization of some of the cells of the nucellar beak was observed (*fig. 5a*). Two transverse rings of cells, several layers beneath the epidermis, begin to disorganize; later the epidermis becomes involuted between the disorganized rings, resulting in a deep groove around the nucellus. The epidermal cells remain very vigorous in appearance, and when the endosperm develops into this region the groove disappears. The cause and the significance of this disorganization and of the temporary involution of the epidermis cannot be suggested.

EMBRYO

LOTSY has described the entrance of pollen tubes into the embryo sac, the fertilization of the free eggs, the excessive elongation of the fertilized eggs to form suspensors, and the cutting-off of the embryonal cells at the tip of the suspensor. Later stages in the development of the embryo have been described by BOWER,[4] but the intermediate stages have not been observed. Fortunately our material from the Philippines contained them, and revealed an unexpected situation.

When the endosperm has become fully developed, its peripheral

[4] BOWER, F. O., The germination and embryology of *Gnetum Gnemon*. Quart. Jour. Micr. Sci. 22:278–298. *pl. 25*. 1882.

region contains a tangle of long, tortuous, and branching suspensors (*figs. 6, 7*), which are difficult to trace. During the formation of a suspensor by a fertilized egg, free nuclear division occurs, resulting in a few nuclei (four in *fig. 7*) distributed along the suspensor. Usually between these nuclei transverse walls are formed by the development of a cleavage plate from the wall of the suspensor. A cell at the tip of the suspensor is cut off in the same way, and contains one of the free nuclei, which becomes associated with numerous starch grains (*fig. 7*).

In this terminal embryonal cell free nuclear division continues (*figs. 8, 9, 10*), accompanied by cleavage walls, until a multicellular embryo is formed. In *figs. 9* and *10* it will be observed that this cleavage apparently continues until uninucleate cells are produced; and in our material this stage is reached first by a group of cells on one side of the embryo. It could not be determined whether this group holds any relation to a body region or not.

It has been supposed that in the embryogeny of Gnetum the preliminary stage of free nuclear division, common to other gymnosperms, had been eliminated; and that the first nuclear division was accompanied by wall formation, as in angiosperms. In *Gnetum Gnemon*, however, free nuclear division not only characterizes the proembryo, but also the early stages of the embryo. The case may be compared to that of Ephedra,[5] in which free nuclear division within the fertilized egg results in eight independent proembryonal cells, each of which continues free nuclear division and develops as a suspensor, which by a cleavage wall cuts off the terminal embryonal cell. In Gnetum the suspensor is formed by the fertilized egg instead of by a proembryonal cell, but the number of free nuclei formed by the egg in each case is approximately the same.

INTEGUMENTS

The mature seed of *Gnetum Gnemon* gives an opportunity to compare the integument and testa with those of other gymnosperms. *Fig. 6a* shows the seed slightly stalked within the so-called "perianth," which is fleshy. Two integuments are evident, and they develop in

[5] LAND, W. J. G., Fertilization and embryogeny in *Ephedra trifurca*. BOT. GAZETTE 44:273–292. *pls. 20–22*. 1907.

basipetal succession. The inner one extends above to form the elongated micropylar tube, and at the maturity of the seed completely invests the nucellus (at this time replaced by the endosperm) as a papery layer. The outer integument becomes differentiated into an outer fleshy layer (white in the figure) and an inner stony layer (black in the figure), the latter completely investing the seed, the former being chiefly developed in the region of the nucellar beak. Two sets of vascular strands are present, the outer set traversing the fleshy layer of the outer integument, the inner set traversing the inner integument.

In Cycadophytes, Ginkgoales, and Coniferales, a single integument becomes differentiated into a testa of three layers: outer fleshy, stony, and inner fleshy. In Gnetum the same three layers are present, but the inner fleshy one has become differentiated in ontogeny as a separate integument. In all cases, this innermost layer finally forms a papery lining of the stony layer. Among the Pinaceae the outer fleshy layer is present in the integument, but it does not develop into the extensive pulpy investment that characterizes the Cycadales, Ginkgoales, and Taxaceae, a fact which is probably associated with the close investment of the seeds by the scales.

The variation in the distribution of the vascular strands among these layers is interesting. Among the more primitive Cycadofilicales and Cordaitales, in which the nucellus is relatively free from the integument, the outer set of strands traverses the outer fleshy layer and the inner traverses the peripheral tissue of the nucellus. In other Cycadofilicales and Cordaitales, however, and in Cycadales in which the nucellus and integument are free only in the region of the nucellar beak, the inner set of vascular strands traverses the inner fleshy layer of the integument; and this is the condition in Gnetum, except that this layer has become differentiated as an inner integument. In Ginkgoales the outer set of strands (belonging to the outer fleshy layer) is suppressed; in Taxaceae the inner set (belonging to the inner fleshy layer) is suppressed; and in Pinaceae both are suppressed.

MALE GAMETOPHYTE

It was a disappointment that the development of the male gametophyte was not secured, for it is only known among Gnetales in Ephedra, in which it has been described by LAND.[6] The development of the tetrad was observed; and although the early anaphase of the first mitosis was not available for the counting of chromosomes, the late prophase of this mitosis and the anaphase of the second mitosis showed clearly that the chromosome numbers are 12 and 24.

SUMMARY

1. The "antipodal tissue" described by LOTSY as occurring in *Gnetum Gnemon* at the fertilization stage is a sharply differentiated nutritive tissue developed in the nucellus beneath the embryo sac, which at this stage contains only free nuclei, as described for other species of Gnetum.

2. Embryo formation begins with an excessive, suspensor-like elongation of the fertilized egg, accompanied by free nuclear division and cleavage walls; and the continuation of free nuclear divisions and cleavage walls in the embryonal cell until a multicellular embryo is formed.

3. The endosperm encroaches upon the tissue of the nucellar beak with some irregularity, an irregularity which reaches its extreme expression in Torreya, with its so-called "ruminated" seeds.

4. The inner integument of the ovule is the morphological equivalent of the "inner fleshy layer" of the single integument of other gymnosperms; and the occurrence of two sets of vascular strands is a relatively primitive condition, which has been departed from by Ginkgoales and Coniferales.

5. The chromosome numbers are 12 and 24.

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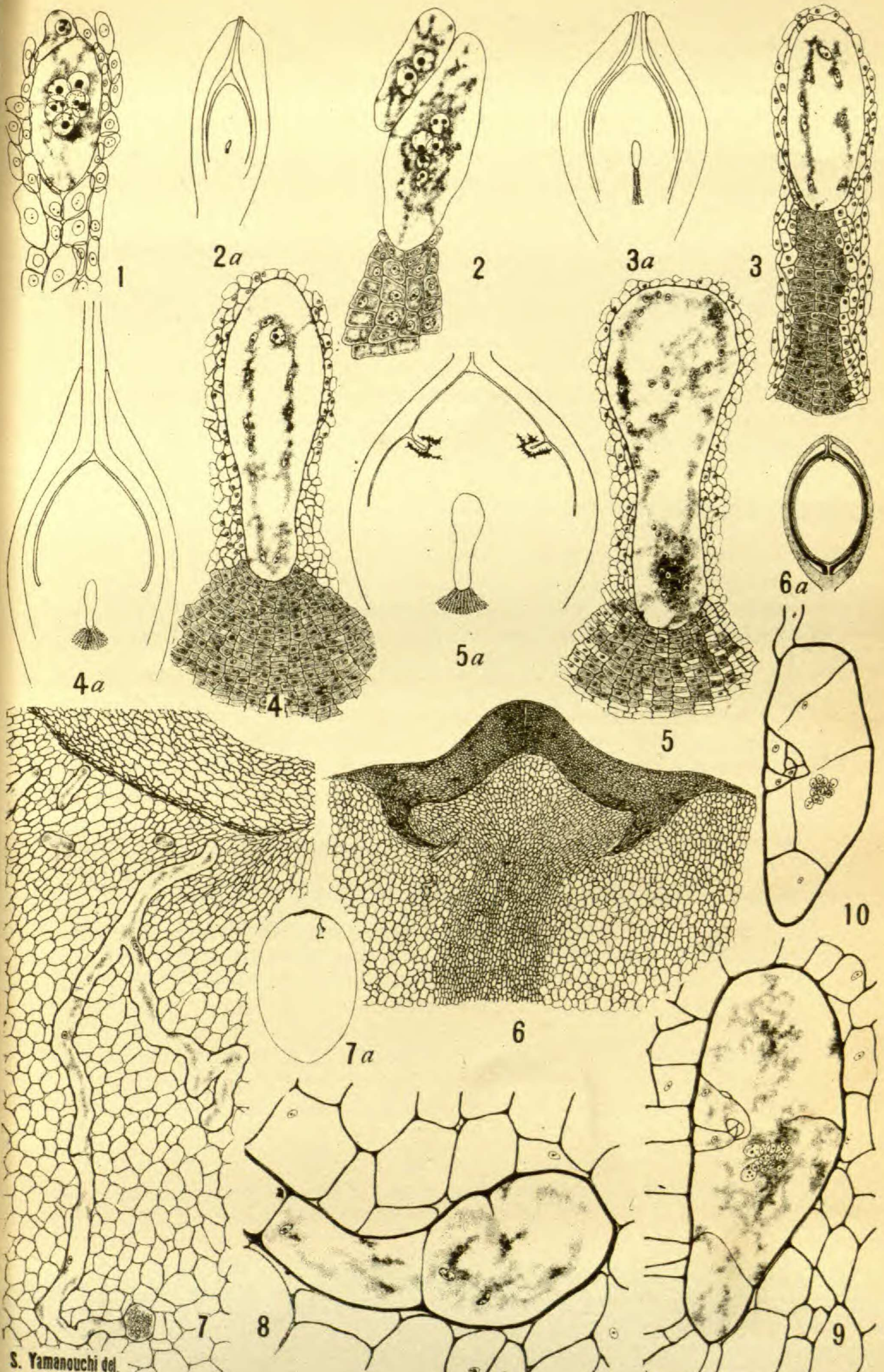
EXPLANATION OF PLATE VII

FIG. 1.—Embryo sac at an early stage, with centrally placed group of eight free nuclei; the cell above, with a large nucleus, is another embryo sac. ×500.

FIG. 2.—Somewhat later stage of embryo sac (all the nuclei not included), showing the beginning of the formation of the pavement tissue; a second embryo sac is also shown. ×500.

FIG. 2*a*.—An ovule at an early stage, showing the two integuments and the relation of the pavement tissue and embryo sac to the nucellus at the stage shown in *fig. 2*. ×40.

6 LAND, W. J. G., Spermatogenesis and oogenesis in *Ephedra trifurca*. BOT. GAZETTE 38:1–18. *pls. 1–5*. 1904.



COULTER on GNETUM